THE ORIGIN OF THE ERECT CELLS IN THE PHLOEM OF THE ABIETINEAE

M. A. CHRYSLER

(WITH TWELVE FIGURES)

In the course of a comparative study of the phloem of conifers, certain facts have come to light which seem to demonstrate the origin of the cells found on the margins of medullary rays in their course through the phloem, the so-called "erect cells" of the rays. These cells are distinguished from the other cells of a ray by their elongation in the vertical direction, their almost total lack of starch, and their possession of sieve areas. Of these three criteria, the third is the final one, while the other two afford presumptive evidence of the nature of cells which may be found at the margin of a ray. Such cells are characteristic of the following genera: *Pinus*, *Picea*, *Larix*, *Pseudotsuga*, *Cedrus*, *Tsuga*, *Abies*, and also occur sporadically in *Juniperus* and *Thuja* (7).

In pitting and contents these cells strongly suggest a homology with sieve tubes, as STRASBURGER (5) has pointed out. The same author observed that like sieve tubes these cells lose their contents and collapse as the season advances, while the central cells of a ray enlarge and become laden with starch. In his paper on ray tracheids THOMPSON (7) goes so far as to say that the erect cells are "virtually ray sieve tubes," and, from the fact that marginal cells of both xylem and phloem portions of a ray arise from the same cambial cell, argues that the marginal cells in the xylem are tracheary in origin. The assumption that erect cells are sieve tubes in nature or origin is to be put to a test in the present paper.

There are several possible origins for these cells. They may, like the ordinary (prone) cells of a ray, merge into the pericycle, or they may simply be prone cells which have taken on a new function and acquired a special shape, or they may be cells distinctly added or applied to a ray already existing in a simple condition, that is, constituted only of prone cells.

An obvious method of attaining a solution of the question is

suggested by the fact that young rays altogether lack erect cells. This may readily be observed in a seedling or in the early layers of growth of a stem or root. Attention has naturally been directed to the stage of growth at which the erect cells make their appearance, and a search has been made among the different organs of the plant for regions where a primitive condition would be likely to appear.

The labors of SCOTT and of JEFFREY have shown that the reproductive axis is one of the places where primitive conditions frequently persist. It has already been shown (**3**) that ray tracheids are absent from the megasporangiate cone of *Pinus;* it may now be added that a search fails to reveal the presence of erect cells in the phloem region of the cone in *Pinus*, *Picea*, and *Abies*. Accordingly, the reproductive axis yields no results which apply to the present question. The leaf also is not well adapted to the present study, on account of the limited development of the phloem. The seedling, which has been studied with such advantage in other cases, has yielded results of much interest in the present instance, especially when the root is the part used. With the roots of seedlings have been compared young regions of roots of more mature plants.

While *Pinus* has been made the basis of the present study, material of all the genera of Abietineae except *Keteleeria* has been available. Representatives of the hard and soft pines (*P. resinosa* and *P. Strobus*) and of species of *Picea*, *Larix*, *Tsuga*, and *Abies* are abundant around Orono, while material of *Cedrus* and *Pseudolarix* has been kindly supplied by Professor E. C. JEFFREY of Harvard University, and *Pseudotsuga* by Mr. F. D. DAVIS of Missoula, Montana. The pines of this vicinity have been supplemented by *P. rigida* collected by Mr. L. L. WOODS of Wells, Maine, and *P. cembroides*, *P. edulis*, and *P. aristata* through the kindness of Dr. FORREST SHREVE of the Desert Laboratory, Tucson, Arizona. To each of these gentlemen I wish to express my obligation. In providing material the difficulty has been to secure the necessary stages; if the roots are too young the erect cells are absent, if too old the cells have collapsed to such an extent as to obscure their relationships. An age of about four to six years has been found to include the significant stages in *P. Strobus*, but older roots must be

used in the case of nut pines. Whenever possible, the material was preserved in a mixture of corrosive sublimate and picric acid dissolved in 30 per cent alcohol, desilicified in hydrofluoric acid, and imbedded in celloidin. For rendering the sieve areas visible, hematoxylin, according to EHRLICH or especially HAIDENHAIN, proved useful, while a counter stain of safranin was generally employed. RUSSOW'S callus reagent was occasionally of use.

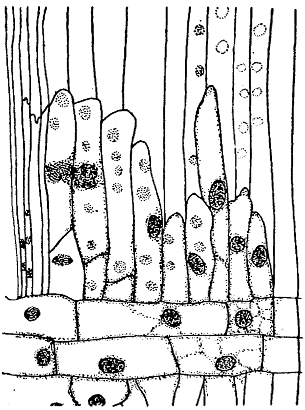

FIG. 1.—*Pinus Strobus*, 6-year root: radial section through phloem in region of a ray, showing long erect cells with sieve pores, and a double nucleus in two cases; the cambial region in this and the other figures lies to the right; ×275.

The appearances in *P. Strobus* will be first described. Fig. 1 represents in radial view the phloem region of a ray from the 6-year region of a root of a seedling of this age. The height of the erect cells is here considerably greater than is usually figured, for example, by STRASBURGER (**5**) and also copied in his *Textbook* (**6**), and such elongated cells are frequent in young roots. They suggest a close relation of these cells to sieve tubes, as do also the sieve areas which are clearly visible in this section. The cambial region of this and all the other figures illustrating this paper lies to the right. Two of the older erect cells show a doubling of the nucleus, which habit, as STRASBURGER points out (**5**, p. 68), is characteristic of young sieve tubes. Immediately to the left of these binucleate cells the crushed and empty older marginal cells are to be seen.

Fig. 2 shows the region bordering on a medullary ray from an 8-year root of *P. cembroides*. On the phloem side of the cambium are to be seen a number of prismatic cells arranged in several series in the radial plane. This layer of cells is intercalated between radial rows of sieve tubes. In some of these cells sieve plates are visible, while the protoplasm becomes more scanty as the older region of the phloem is approached. In other sections such cells may be seen to lose their cytoplasm and nucleus (frequently after

amitosis), and finally become completely collapsed during their second year, after the manner of sieve tubes. On the xylem side a few short tracheids are cut off, but their walls are thin, and they can be traced for only about the width of two tracheids.

Cells in radial groups are frequent in the phloem of young roots in all the species of *Pinus* which have been studied, and their occurrence has been recorded by STRASBURGER (**5**), who states that they are characteristic of the young regions of the plant, before the tangential rows of phloem parenchyma cells become established. The present investigation goes to show that the radial groups of cells are not only more abundant in the young regions, but that they are more common in young roots than in stems. It should be stated, however, that the cells of the radial rows do not for the most part correspond to phloem parenchyma, as will presently appear. In many cases the cambium for a period of one or more years cuts off no cells on the xylem side, but generally from the median region of such radial groups a narrow medullary ray is developed, as is clearly shown in fig. 3, which is drawn from a 5-year root of *P. resinosa*. Cambial activity dwindles until it is confined to the median region of the radial group, and a medullary ray one cell in height is formed. It will be noticed that in the xylem region this ray consists of tracheids, though cases occur where the ray cells first formed are tracheids and the later ones are parenchymatous, as shown by the pitting. Thus a tracheidal ray may turn into a parenchymatous one, but the reverse seems to happen seldom if ever. Attention has been called to this point by THOMPSON (**7**). The initial

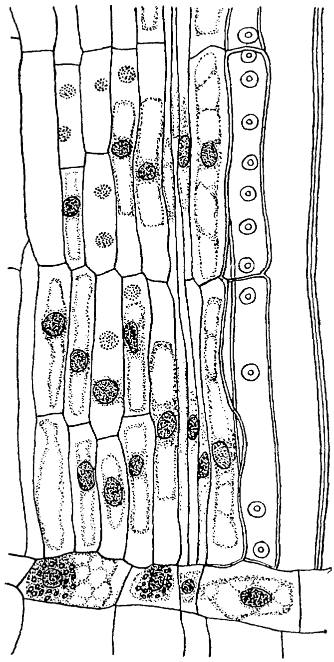

FIG. 2.—*P. cembroides*, 8-year root: radial group of cells provided with sieve pores; at the right the cambium gives rise to a few thin-walled short tracheids; $\times 275$.

stages in the development of the xylem portion of such a ray have been observed in numerous instances, and one is represented in fig. 4, from a 7-year root of *P. aristata*. It will be seen that the narrow ray ends on the xylem side in a somewhat pointed cell. This method of formation of a narrow ray differs from the one described by THOMPSON in that the initial activity seems here to be on the phloem side, and the narrow ray is wholly independent of

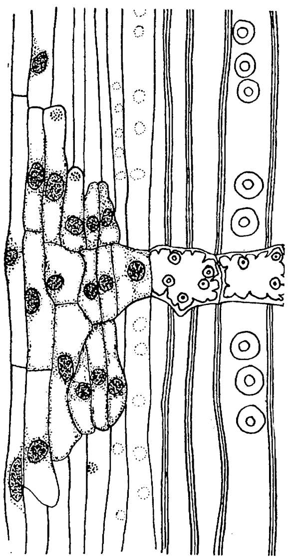

FIG. 3

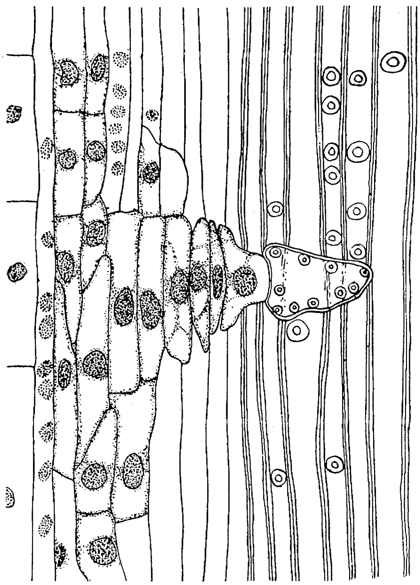

FIG. 4

FIGS. 3, 4.—Fig. 3, *P. resinosa*, 5-year root: radial plate giving rise to a narrow ray; ×275; fig. 4, *P. aristata*, 7-year root: beginning of the production of a narrow ray; ×275.

transitional elements on the xylem side. Instead of giving rise to a single narrow ray, a radial group may be lined up with two or three narrow rays, which, however, consist at first wholly of tracheidal cells. We have here a prolific source of new, that is, secondary rays in the young root. I have observed the formation of parenchymatous rays in the same manner in *Juniperus*.

In fig. 5, from the same root as fig. 3, is seen what frequently takes place when one of the radial groups adjoins a medullary ray.

The cells in question apply themselves to the side of the ray, and as cambial activity in the radial group slackens, this activity is restricted to the border of the ray, thus changing the ray from a simple one consisting only of parenchymatous cells to a ray provided with marginal cells. With fig. 5 may now be compared fig. 1, which shows the same mode of origin for the marginal cells of the phloem region of the ray in *P. Strobus*. Observations have been made on representatives of the hard and soft pines, including the nut and foxtail pines, and appearances similar to fig. 5 have been found in all, when roots of appropriate age were examined.

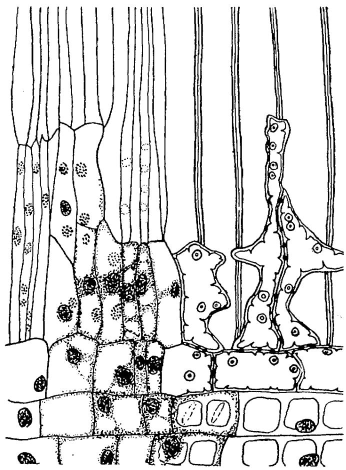

FIG. 5.—*P. resinosa*, 5-year root: a radial plate is in contact with a medullary ray, producing a row of marginal cells; $\times 275$.

Regions where two rays lie near one another in the vertical plane present appearances which may readily be explained in the light of what has been said of the radial plates. Fig. 6 shows such a region in a 6-year root of *P. Strobus*. In the upper part of the figure is a ray which on one margin is destitute of erect cells, and on the other (lower) margin is provided with a fringe of much elongated cells which soon merge into a group of prismatic cells forming a radial plate of tissue by means of which the ray shown is connected with another which lies below the area covered by the figure. These cells of the "radial plate" show precisely the same histological features as those previously described, such as the sieve areas which are here shown. At the extreme left of the figure is a row of phloem parenchyma cells, which are easily distinguished from the cells of the radial plate by the starchy contents and swollen shape of the former.

Further transition stages are shown in fig. 7, from a 5-year root of *P. Strobus*, which represents cases where two rays are closer than those in fig. 6. Almost from the time of their formation from

cambial cells the erect cells of the two rays come into contact, and soon merge into the condition where a single cell spans the space between the rays. As before, such cells lose their contents and become greatly compressed in the radial direction.

Fig. 8, from the same root as fig. 7, represents a case where two rays are practically in contact. A cambial cell is seen to give rise on the phloem side to pairs of cells which appear to have earlier been undivided, as seen in the extreme left of the figure; the nature

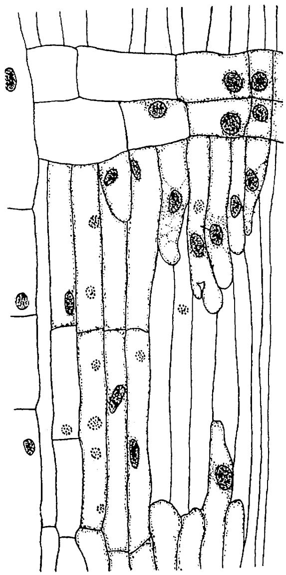

FIG. 6

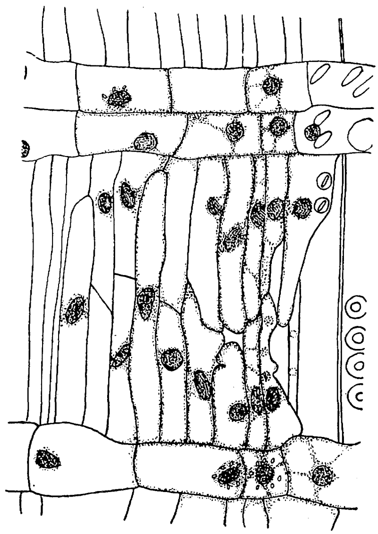

FIG. 7

FIGS. 6, 7.—Fig. 6, *P. Strobus*, 6-year root: region between two rays, with radial plate merging into erect cells; ×275; fig. 7, 5-year root: two rays nearer than in fig. 6.

of these cells is shown by the sieve areas as before. On the xylem side the cambial cell has given rise to irregular shaped tracheids which are evidently of the kind described by THOMPSON (7), and by him regarded as transitional forms between ray tracheids and regular tracheids of the xylem.

It is altogether probable that the prismatic cells lying between two neighboring rays, as shown in figs. 6–8, are of the same nature as the cells of the radial groups, inasmuch as the contents, pitting,

and fate of the cells are the same in both cases. Where two rays are in vertical proximity, it is to be expected that the intervening space will be occupied by one or two cells, but here, as in the case of the radial plates of cells, there is seen a tendency for the cambial activity to be localized in the region of a ray.

Several of the figures illustrate a point which must be emphasized, namely, a ray uniformly begins its course in the outer phloem as a simple structure consisting exclusively of "prone" cells which at certain times of year contain abundant starch, and

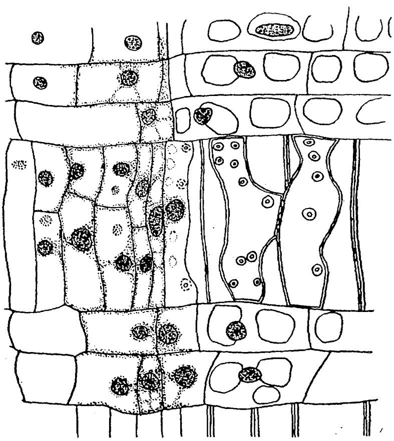

FIG. 8

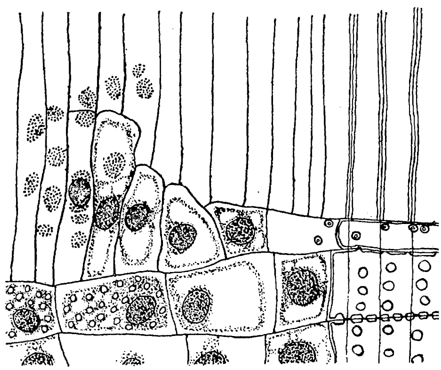

FIG. 9

FIG. 8, 9.—Fig. 8, *P. Strobus*, 5-year root: two rays almost in contact; fig. 9, *P. aristata*, 7-year root: origin of erect cells by cutting off a cell from the end of a sieve tube; ×275.

only after cells of a radial group become applied to its margin does the ray come to have a border of "erect" cells. Such a border is usually added to only one edge of the ray at a time, though cases occur in which borders are added to both edges simultaneously.

A slightly different mode of origin of the erect cells has been observed in a number of cases. One of these is shown in fig. 9, from a 7-year root of *P. aristata*. Beginning at the left it will be seen that the ray is not provided with a border of erect cells, but that sieve tubes come in contact with the ray. A little farther to the right an elongated cell provided with sieve pores is cut off from a

sieve tube, and immediately a border to the ray is produced, with the erect cells as usual coterminous with marginal tracheids. Similar instances have been found in other species of *Pinus*, and the transition from sieve tube to erect cell is not always as abrupt as in the case figured. Since the cells of the radial rows are sieve tubes in all respects save length and the nucleus, the occasional formation of an erect cell by cutting off a segment from a sieve tube does not seem surprising. In fact it does not seem necessary to assume an absolutely uniform mode of origin for the erect cells, in view of the fact that a certain cambial cell may be giving rise to ray tracheids, but for some reason suddenly cut off a parenchyma cell instead. Similarly a row of ray cells of the phloem may at first be albuminous, but later-formed members of the row may contain starch. We are interested here, however, in the question as to the evolutionary origin of the erect cells, rather than in occasional modes of origin.

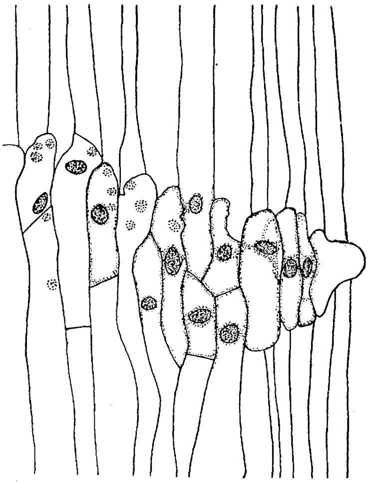

FIG. 10.—*P. Strobus*, 4-year root: sieve tubes have cut off cells from their ends, resulting in production of a ray; ×275.

An interesting mode of formation of a ray, showing the tendency of sieve tubes to cut off a cell from the end, has been observed a few times. An example of this mode, from a 4-year root of *P. Strobus*, is represented in fig. 10. In this case the habit of cutting off a cell from the end of a sieve tube has become so well established that a narrow ray has originated, as seen in the cambial region at the right. This process is entirely similar to the mode of origin of erect cells described in the preceding paragraph.

Although the most significant results have been obtained from a study of the young root, the stem of *Pinus* has also been examined to some extent, but the mode of origin of the erect cells in this case was so indefinite compared with what may be seen in similar regions

of the root that the study was soon discontinued. THOMPSON'S statement with respect to the ray tracheids may be aptly quoted in this connection (**7**, p. 108): "The root is admittedly more conservative than the stem. Accordingly in the latter the evolutionary processes are not so well represented. There is a hurrying over of the early stages, so that an actual series can rarely be observed."

Examination of the stem of a number of seedlings of *P. Strobus* and *P. resinosa* showed that (1) the rays in both the xylem and the phloem regions were without marginal cells for several years; (2) the marginal cells as a rule make their appearance slightly earlier in the phloem than in the xylem. This feature is shown clearly in roots as well; in fig. 11, from a 5-year root of *P. resinosa*, is a triangular cambial cell which has cut off a succession of erect cells, while as yet no cells have been cut off on the xylem side. Fig. 7 shows the same feature, and with it may be compared the condition represented in fig. 4. Addition of the marginal cells does not begin in all or many of the rays simultaneously, but after the plant is two to three years old a few of the rays acquire the border.

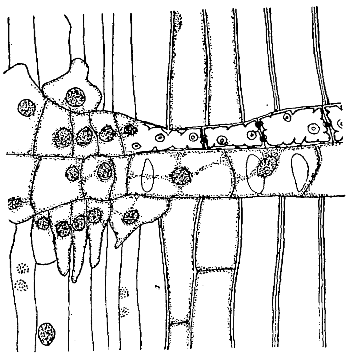

FIG. 11.—*P. resinosa*, 7-year root: the cambial cell on the lower side of the ray cuts off cells on the phloem side but not on the xylem side; ×275.

With respect to the remaining genera of Abietineae, only a few species have been studied with any approach to thoroughness, but enough has been done to show that many of the features figured in this paper occur in *Picea*, *Larix*, *Tsuga*, and *Abies*. The same radial groups of cells occur, and the phenomena seen where two rays are vertically contiguous may sometimes be seen. For instance, fig. 6 might be almost duplicated from a young root of *Tsuga canadensis*.

The genus *Abies* presents points of interest, on account of the fact that most species lack marginal cells in the xylem, though they are generally present in the phloem. THOMPSON points out that the erect cells are "never in line with the parenchyma cells of the ray,

but always above or below them. Often they are in line with two or three degenerating cells on the wood side" (**7**, p. 112). The conclusion seems justified that *Abies* represents a genus which has descended from ancestors which possessed ray tracheids, and is less primitive than *Pinus*. In spite of this, it may sometimes be made out in young roots of *Abies balsamea* that the erect cells have the same mode of origin as has been described for *Pinus*. Fig. 12 illustrates this point, as well as the fact that the triangular cambial cell gives rise to erect cells but not to ray tracheids. In many instances the shadowy remains of marginal tracheids appear in these root sections.

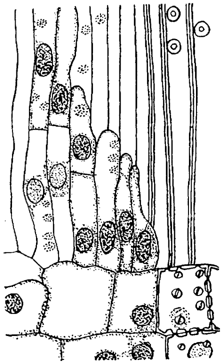

FIG. 12.—*Abies balsamea*, 8-year root: the erect cells have the same mode of origin as in *Pinus;* the cambial cell at margin of the ray cuts off erect cells but no ray tracheids; ×275.

With this persistence of marginal cells in the phloem may be compared their earlier appearance in the phloem than in the xylem, as seen in seedlings of *Pinus*. Just why a cambial cell should be more apt to cut off segments on the phloem side than on the xylem side is hard to say; since the supply of food is on the phloem side, unequal nutrition may be the cause at work. But it is possible that we must consider the phloem to be a more conservative region than the xylem, in which case the observation has interest from the phylogenetic standpoint. The latter view of the case is supported by my observations on *Juniperus*, in which genus PENHALLOW (**4**) found ray tracheids occurring sporadically. If a radial section of *J. communis* is cut so as to include phloem as well as xylem, it is seen that where ray tracheids occur near the cambium they are coterminous with erect cells. But in the root of *J. virginiana*, in which species ray tracheids have not been reported, a number of instances have been found where the phloem portion of a ray is provided with a border of well marked erect cells, ending at the cambium in a blunt cell, reminding one of the appearances in *Abies balsamea*. Judging by the leaves, *J. communis* is a more primitive species than *J. virginiana*, hence we are prepared to find ancestral features per-

sisting in the former which have been lost in the latter, that is to say, marginal cells occur in the xylem and phloem of *J. communis*, while they are restricted to the phloem of *J. virginiana*. If, as recent studies indicate, the Cupressineae are derived from large-leaved conifers such as the Abietineae, the persistence of marginal cells in the phloem after they have disappeared from the xylem must be interpreted as the retention of an ancestral feature, which amounts to saying that the phloem is a comparatively conservative region.

The genus *Cedrus* shows in a marked way the belated appearance of the ray tracheids compared with the erect cells, but a detailed consideration of *Cedrus* and *Pseudolarix* is reserved for separate treatment in a future paper.

The choice of *Pinus* for detailed study has been made on the basis of its probably primitive nature, in the light of recent paleontological discoveries. If the origin of the erect cells is established for *Pinus*, it holds for the other genera of Abietineae, though they may not so clearly show the formative stages. Within the genus *Pinus* it appears from the work of BAILEY (1) that the nut and foxtail pines are to be regarded as the most primitive, hence *P. edulis*, *P. cembroides*, and *P. aristata* from Arizona were studied with especial interest. Although difficulties were experienced in judging the ages of roots, and seedlings were not available, the material showed the same appearances as had been observed in the eastern pines, as several of the figures indicate. One point was established which forms an additional argument for the primitive nature of these species, namely, the marginal cells in the xylem make their appearance considerably later than in the eastern pines investigated. Since marginal cells are absent from the reproductive axis and from the early rings of growth in stem and root, and particularly because they are not found in the older Pityoxyla (**3**), they are regarded as of comparatively recent introduction, and it follows that those species in which they appear late in development are to be considered primitive, unless other evidence indicates the contrary. In respect to the time of appearance of the marginal cells, the nut pines and certain of the hard pines, such as *P. rigida*, stand at opposite extremes, according to the limited study which this point has received.

Doubt has been cast by BAILEY (**2**) on THOMPSON'S theory as to the origin of ray tracheids (**7**), on the ground that the roots used were probably wounded. With this criticism in mind, the material used in the present investigation has been carefully examined for evidences of wounds. Though the possibility of such is not denied, it may be said that the soundest and straightest pieces were selected for study, and no indications of wounds were seen in the sections.

It may be fairly considered that the relation of erect cells to sieve tubes is established, first because of the occurrence in primitive regions of the plant of erect cells so greatly elongated as to be eight times as long as wide, secondly because of their containing much protoplasm but no starch, thirdly because they possess sieve pores, fourthly because they eventually lose their contents and collapse, frequently after the nucleus has divided amitotically. In all of these respects they resemble sieve tubes. To this may be added that they are sometimes cut off from the end of a sieve tube. They are in fact sieve tubes except for possession or rather retention of a nucleus, for the matter of length is a minor one in view of the occurrence in the young root of much elongated erect cells, and the great variation in length of sieve tubes in mature plants of different species, short tubes occurring for instance in the classic case of *Cucurbita*.

In accounting for the origin of erect cells, an examination of admittedly primitive regions of the plant seems to show that they owe their presence on the edge of an originally simple ray to the adhesion, so to speak, of a group of prismatic cells lying in the radial plane. It has been shown that such radial groups are frequent in young parts of the plant, especially the root, and that they occur among radial rows of sieve tubes. But these radial plates of cells represent a passing phase; soon cambial activity in a plate dwindles and becomes restricted to one or a few cells, in which event there are two possibilities: (1) one or more narrow rays may arise, consisting on the phloem side of albuminous cells and on the xylem side of horizontal tracheids, or (2) the cambial activity may become localized at the edge of a ray already existing, producing a series of marginal cells, the so-called erect cells. The earlier members of a

series of these cells are more elongated than the ones formed later, but all possess the same histological features and undergo the same fate. Similar in form and contents are the cells which span the space between two vertically contiguous rays, and which by fission and shortening give rise to a row of erect cells on each of the two rays.

Since the cells of the radial rows occur principally in the young plant, and have the same contents as sieve tubes, with the exception of the nucleus, it is a tempting theory to consider them the forerunners of sieve tubes, the primary sieve cells which undergo evolution in two directions: (1) lengthening out and losing the nucleus so as to produce sieve tubes, and (2) shortening as they become applied to medullary rays and become converted into erect cells or as they give rise to rays independently. But such a theory must stand on the evidence of a comparative study of phloem of vascular plants, and the evidence is not yet at hand.

Summary

1. The "erect cells" occurring on the margins of medullary rays in the phloem of most genera of Abietineae do not exist in the young ray, which consists only of ordinary parenchyma.

2. In young roots of *Pinus* the phloem shows certain cells which are essentially short sieve tubes possessing nuclei, occurring in groups in the radial plane. On the xylem side these may merge into one or more narrow rays consisting of tracheids, owing to a diminution and localization of cambial activity.

3. When such a radial group occurs in vertical contact with a medullary ray, cambial activity is sooner or later localized at the edge of the ray, resulting in the production of a border to the ray, such border consisting of sieve cells, which are the erect cells found in mature phloem.

4. Variations of this mode of origin of erect cells occur, such as the cutting of a cell from the end of a sieve tube when it meets the edge of a ray.

5. In young roots and stems marginal cells may make their appearance in the phloem earlier than in the xylem, while in *Abies*

marginal cells have disappeared from the xylem although not from the phloem. This and other observations indicate that phloem is a more conservative region than xylem.

UNIVERSITY OF MAINE
ORONO, MAINE

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